PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 8028-007-WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US04/33260	International filing date *(day/month/year)* 07 October 2004 (07.10.2004)	Priority date *(day/month/year)* 10 October 2003 (10.10.2003)
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 15/85, 15/86, 15/00; C12P 21/00; A61K 38/19 and US Cl.: 435/325, 326, 70.1, 69.1; 514/2		
Applicant MULTICELL TECHNOLOGIES INC.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____ , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 August 2005	01 February 2006 (01.02.2006)
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Form PCT/IPEA/409 (cover sheet)(April 2005)

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Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into English, which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4(a))
- ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1-90 as originally filed/furnished
pages* NONE received by this Authority on
pages* NONE received by this Authority on

☒ the claims:
pages 91-98 as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* 91-98 received by this Authority on 28 October 2005 (28.10.2005)
pages* NONE received by this Authority on

☒ the drawings:
pages 1-17 as originally filed/furnished
pages* NONE received by this Authority on
pages* NONE received by this Authority on

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 3,7,9,19-21,27,28,30,31,33,34,37,43 and 46-61	YES
	Claims 1,2,4-6,8,10-18,22-26,29,32,35,36,38-42,44,45	NO
Inventive Step (IS)	Claims 3,7,21,30,31,34,37,	YES
	Claims 1,2,4-6,8-20,22-29,32,33,35,36,38-61	NO
Industrial Applicability (IA)	Claims 1-61	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)
Please See Continuation Sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 46-61 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description.

The description does not disclose the claimed invention in a manner sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because: the entire description is dedicated to methods of expressing a protein in cell culture. Claims directed to a pharmaceutical composition (claims 51-55) are directed to therapeutic compositions (i.e., pharmaceutical), thus read on *in vivo* therapy. There does not appear to be any teachings in the description for *in vivo* methods of treating a disease.

In vivo methods of treating disease of an animal or a human being is not deemed routine in the art generally. More particularly the modalities of diseases, including liver disease involve pathways and mechanisms that can vary from the mode of treatment to the actual therapeutic/pharmaceutical agent being utilized. For example, injection into an animal/human of a recombinantly produced protein, depending on the secondary/tertiary structure of the protein, can elicit immnotoxicogenic responses in the animal. Furthermore, a given protein may be subject to degradation or removal before any therapeutic effect is conferred. Moreover, the route of delivery for a perceived therapeutic protein may determine beneficial outcome/results. On whole, the level of unpredictability in the art of *in vivo* methods of treating disease with a recombinant/cultured protein is high enough, so that undue experimentation is necessary to practice the claimed methods and to produce and utilize the claimed pharmaceutical compositions. The specification simply does not provide any teaching with respect to the objected to claims, and such an omission is not provided for by evidence in the art.

In the remarks, beginning on page 5, Applicant contends that the claims are fully supported by the specification for *in vivo* therapy. Applicant asserts: a) native human proteins will not be immunogenic; b) there is no evidence that any of the proteins would be subject to degradation or removal; c) many recombinant proteins are now used as *in vivo* therapeutics; and c) the specification teaches how to use the claimed invention for therapy. These arguments have been fully considered but are not deemed persuasive. The claims embrace a method of treating any condition using any protein produced by the methods disclosed in the specification. The proteins are not limited to being human proteins and in many claims are not limited to being plasma proteins. The teachings in the specification with regard to pharmaceutical application are generic and are not sufficient to instruct the skilled artisan in how to treat any given disease with any given protein. The teachings are, in essence, an invitation to the skilled artisan to determine for himself or herself how to use the proteins produced by the method for therapeutic application. Given the vast scope of the claims and the absence of any specific guidance as to how to practice the therapeutic methods claimed, one would clearly conclude that the claims are not fully supported by the description.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

V. 2. Citations and Explanations:
Claims 1,2,4-6,8,10-18,22-26,29,32,35,36,38-42,44,45 lack novelty under PCT Article 33(2) as being anticipated by Kobayashi et al. (ASAIO Journal. 2002; 48:355-59).

Kobayashi teaches expression of proteins in an immortalized human hepatocyte. (e.g., Abstract, pp. 355-6, Materials and Methods). More particularly, the reference teaches expression of three different proteins, i.e., CYP 2C9, CYP 3A4 and p21. (e.g., p. 357, Figures 1-3). Furthermore, the cells are immortalized by transduction with SV40 large T antigen. (e.g., p. 355, col. 2; p. 358, col. 1, Discussion). In addition, the cells are transducted with an additional vector - adenovirus - encoding p21 protein. (e.g., p. 356, col. 1, last ¶, bridging to col. 2). The p21 protein does confer a *therapeutic* effect insofar as it blocks cell cycle progression and induces beneficial changes in the hepatocyte cells. (e.g., p. 358, col. 2, ¶¶ 2-4). Therefore, the cited claims are not novel over the teachings of Kobayashi.

In the remarks beginning on page 5, Applicant argues that the teachings of Kobayashi do not anticipate the claims because: a) Kobayashi *et al.* does not teach that the protein is expressed such that its *in vivo* function is substantially preserved after its isolation; b) the protein expressed would not be isolated from cells or used for therapy; c) the proteins of Kobayashi *et al.* are not glycosylated; and d) Kobayashi *et al.* does not disclose how the proteins can actually be isolated in active and, if necessary, properly glycosylated form.

These arguments have been fully considered but are not deemed persuasive. The arguments seek to distinguish the teachings of Kobayashi *et al.* from the claimed invention based on limitations that do not appear in the claims. The claims require that the protein be expressed such that its *in vivo* function is substantially preserved after its isolation. The claim does not require that the isolation step (c) provide a protein wherein the function of the protein is preserved, but that the protein is expressed in the cell such that it could be isolated in functional form. Therefore, anticipatory art need not actually disclose an isolation method that provides fully functional protein. Furthermore, even if the claims did require isolation of a fully functional and therapeutically useful protein, such isolations are routinely practiced and would be obvious over a teaching of protein expression in a suitable host cell.

Claims 9, 19-20, 27, 28, 43 lack an inventive step under PCT Article 33(3) as being obvious over Kobayashi in view of Lollar (US Patent No. 6,517,830).

Supplemental Box

Additional embodiments are directed to expression of a particular protein in immortalized human hepatocyte cells - Iα Ip, Rb or p53. Kobayashi does not specifically teach expression of Rb or p53 protein, but expression of a *different* protein would entail nothing more than routine experimentation in view of the knowledge in the prior art.

As evidenced by the art, the same vector system utilized by Kobayahsi to transfect hepatocytes and express p21 protein has been utilized to express other proteins. For example, Lollar teaches that many viral vectors including adenovirus can be utilized to express plasma proteins, such as Factor VIII. (e.g., col. 9, last ¶).

Therefore, it would have been obvious to modify the adenovirus as taught by Kobayashi so as to incorporate into the vector a nucleic acid that encodes an Iα Ip related protein, or Rb or p53, so as to achieve the benefit extending the range of proteins that can be expressed in hepatocyte cells. In addition, it would entail nothing more than routine experimentation and standard techniques in molecular biology to modify a target protein to further comprise a cleavable tag, which could be used to achieve the added benefit to either purify the expressed protein or to measure the expression levels.

In the remarks, page 9, Applicant argues that the claims are not obvious over the art because Lollar *et al.* does not teach expression of proteins in hepatocytes but in liver endothelial sinusoidal cells. This argument has been fully considered but is not deemed persuasive because Kobayashi *et al.*, not Lollar *et al.*, is relied upon for the teaching of protein expression in hepatocytes. Furthermore, adenovirus is routinely used to express proteins in hepatocytes. Therefore, there is no question as to whether an adenoviral vector could be used to express a protein in hepatocytes.

Claims 32 and 33 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Lucas et al. (Nuc. Acids Res. 1996; 24:1774-79)

Additional embodiments are directed to expression of Iα Ip in eukaryotic cells other than human hepatocytes, such as CHO cells. Expression of Iα Ip would involve nothing more that remedial steps so as to construct a vector encoding an Iα I protein, similarly to expressing any recombinant protein. Furthermore, expression of recombinant proteins in eukaryotic cells, such as CHO cells is well known in the art. For example, Lucas teach high-level production of recombinant proteins in CHO cells utilizing an expression vector.

Therefore, it would have been obvious to modify a vector to express a protein of interest such as an Iα Ip and transduce eukaryotic cells commonly utilized such as CHO cells at taught by Lucas, to express recombinant proteins of interest.

In the remarks, page 9, Applicant contends that the claims are not obvious over the art because the vector used in Lucus *et al.* is not used in the cells of the invention and there is not sufficiently high probability of success for expression of proteins normally expressed in hepatocytes in cells such as CHO cells. This argument is not deemed persuasive because the cell of the rejected claims is limited only to being other than a hepatocyte. Furthermore, the art of protein expression is highly developed and CHO cells are routinely used in the art to express heterologous proteins. In view of the well-characterized nature of the art, there is clearly sufficiently high probability of successs in practicing the claimed invention in CHO cells.

We claim:

1. A method of using immortalized human hepatocyte cells to produce a protein comprising the steps of:

(a) providing an immortalized human hepatocyte cell that includes DNA that encodes and can express a protein;

(b) culturing the immortalized hepatocyte cell under conditions in which a gene or genes encoding the protein are expressed so that the protein is produced and processed in the immortalized hepatocyte cell; and

(c) isolating the processed protein from the immortalized hepatocyte cell; wherein the protein is expressed such that the protein is processed and glycosylated, if necessary, so that its *in vivo* function is substantially preserved after its isolation.

2. The method of claim 1 wherein the protein is a plasma protein that is naturally produced by human hepatocytes.

3. The method of claim 1 wherein the protein is a protein that is not naturally produced by human hepatocytes.

4. The method of claim 3 wherein the protein is a mutein of a protein that is normally produced by human hepatocytes.

5. The method of claim 1 wherein the protein is a therapeutic protein.

6. The method of claim 5 wherein the therapeutic protein is a therapeutic plasma protein.

7. The method of claim 6 wherein the protein is selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH), α-1-antitrypsin, and a growth factor.

8. The method of claim 6 wherein the protein is selected from the group consisting of muteins of Factor VIII, muteins of Factor IX, muteins of human growth hormone, muteins of α-1-antitrypsin, and muteins of a growth factor.

9. The method of claim 1 wherein the protein is an IαIp protein.

10. The method of claim 1 wherein the protein is a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

11. The method of claim 1 wherein the protein is a mutein of a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

12. The method of claim 1 wherein the protein is expressed by a gene that occurs naturally in the hepatocytes, and expression of the naturally-occurring gene encoding the protein is enhanced by introduction of a high-level promoter into the hepatocytes.

13. The method of claim 1 wherein expression is enhanced by introducing multiple copies of the gene encoding the protein to be expressed, a subunit of the protein to be expressed, or a precursor of the protein to be expressed via the use of one or more recombinant vectors that include: (1) the gene encoding the protein to be expressed, a subunit of the protein to be expressed, or a precursor of the protein to be expressed; and (2) at least one control element affecting the transcription of the gene, the control element being operably linked to the gene.

14. The method of claim 13 wherein the recombinant vector is selected from the group consisting of SV40-derived vectors, murine polyoma-derived vectors, BK virus-derived vectors, Epstein-Barr virus-derived vectors, adenovirus-derived vectors, adeno-associated virus-derived vectors, baculovirus-derived vectors,

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herpesvirus-derived vectors, lentiviral-derived vectors, retrovirus-derived vectors, alphavirus-derived vectors, and vaccinia virus-derived vectors.

15. The method of claim 14 wherein the vector incorporates one or more reporter genes.

16. The method of claim 1 wherein the expressed protein is secreted from the cell into the surrounding culture medium.

17. The method of claim 1 wherein the protein is glycosylated.

18. The method of claim 1 wherein the protein is processed post-translationally.

19. The method of claim 1 wherein the protein is expressed in a form wherein it is fused to a cleavable tag.

20. The method of claim 19 wherein the cleavable tag is selected from the group consisting of glutathione *S*-transferase, the MalE maltose-binding protein, and a polyhistidine sequence.

21. The method of claim 1 wherein the protein comprises at least two different subunits, and wherein the immortalized hepatocyte cell is transformed or transfected with at least two vectors, each vector including: (1) DNA including at least one gene that encodes at least one subunit of the protein; and at least one control element operably linked to the DNA encoding at least one gene that encodes the subunit of the protein.

22. The method of claim 1 wherein the immortalized human hepatocyte cell is virally immortalized.

23. The method of claim 22 wherein the hepatocyte is immortalized by transformation or transfection with substantially pure simian virus (SV40) DNA.

AMENDED SHEET

24. The method of claim 23 wherein the substantially pure SV40 DNA encodes large T and small t antigens (Tag).

25. The method of claim 1 wherein the immortalized human hepatocyte cell is derived from primary cryopreserved human hepatocytes.

26. The method of claim 1 wherein the hepatocyte includes tumor-suppressor-encoding DNA such that substantially pure DNA encoding tumor suppressor can be isolated and purified from the hepatocyte.

27. The method of claim 1 wherein the hepatocyte includes DNA encoding Rb such that substantially pure DNA encoding Rb can be isolated and purified from the hepatocyte.

28. The method of claim 1 wherein the hepatocyte includes DNA encoding p53 such that substantially pure DNA encoding p53 can be isolated and purified from the hepatocyte.

29. The method of claim 1 wherein the hepatocyte is nontumorigenic, has the ability to be maintained in a serum-free medium, and produces plasma proteins.

30. The method of claim 1 wherein the hepatocyte is a hepatocyte of the Fa2N-4 cell line.

31. The method of claim 1 wherein the hepatocyte is a hepatocyte of the Ea1C-35 cell line.

32. A method of using eukaryotic cells, other than human hepatocytes, to produce an IαIp protein comprising the steps of:

(a) providing a eukaryotic cell, other than a human hepatocyte, that includes DNA that encodes and can express proteins forming an IαIp protein complex, the eukaryotic cell having been transformed or transfected with at least one vector that

includes: (1) DNA including at least one gene for a precursor of a protein that is part of an IαIp protein; and (2) at least one control element operably linked to the DNA encoding at least one precursor gene in order to enhance expression of the precursor gene;

(b) culturing the transformed or transfected eukaryotic cell under conditions in which genes encoding proteins forming an IαIp protein are expressed so that an IαIp protein is produced; and

(c) isolating the expressed IαIp protein from the transformed or transfected eukaryotic cell.

33. The method of claim 32 wherein the eukaryotic cell is selected from the group consisting of CHO cells, COS cells, and yeast cells.

34. The method of claim 32 wherein the eukaryotic cell is transformed or transfected with two vectors: (1) a first vector that includes the genes *H3* and *AMBP*; and (2) a second vector that includes the genes *H2* and *H1*.

35. An immortalized human hepatocyte cell that includes DNA that encodes and can express a protein, the immortalized human hepatocyte cell having been transformed or transfected with at least one vector that includes: (1) DNA including at least one gene encoding a protein; and (2) at least one control element operably linked to the DNA encoding the protein in order to enhance expression of the protein, the protein being expressed by the cell such that the protein is processed and glycosylated, if necessary, so that its *in vivo* function is substantially preserved after isolation of the protein.

36. The cell of claim 35 wherein the protein is a protein that is naturally produced by human hepatocytes.

37. The cell of claim 35 wherein the protein is a protein that is not naturally produced by human hepatocytes.

38. The cell of claim 37 wherein the protein is a mutein of a protein that is normally produced by human hepatocytes.

39. The cell of claim 35 wherein the protein is a therapeutic protein.

40. The cell of claim 39 wherein the therapeutic protein is a therapeutic plasma protein.

41. The cell of claim 40 wherein the protein is a therapeutic plasma protein selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH), α-1-antitrypsin, and a growth factor.

42. The cell of claim 40 wherein the protein is a plasma protein is selected from the group consisting of muteins of Factor VIII, muteins of Factor IX, muteins of human growth hormone, muteins of α-1-antitrypsin, and muteins of a growth factor.

43. The cell of claim 35 wherein the plasma protein is an IαIp protein.

44. The cell of claim 35 wherein the protein is a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

45. The cell of claim 45 wherein the protein is a mutein of a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

46. A method of treating a disease or condition comprising the steps of:

(a) providing an active protein produced according to the method of claim 1; and

(b) administering the active protein to a patient suffering from the disease or condition in a therapeutically active quantity to treat the disease or condition.

47. The method of claim 46 wherein the disease or condition is a disease or condition affecting the liver.

48. The method of claim 47 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

49. The method of claim 46 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

50. The method of claim 49 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

51. A pharmaceutical composition for treating a disease or condition comprising:

(a) an IαIp protein produced by eukaryotic cells in a quantity terapeutically effective to treat a disease or condition; and

(b) a pharmaceutically acceptable carrier.

52. The pharmaceutical composition of claim 51 wherein the disease or condition is a disease or condition affecting the liver.

53. The pharmaceutical composition of claim 52 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

54. The pharmaceutical composition of claim 51 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

55. The pharmaceutical composition of claim 54 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

56. A method of treating a disease or condition comprising the steps of:

(a) providing an active plasma protein produced by the method of claim 1; and

(b) administering the active plasma protein to a patient suffering from the disease or condition in a therapeutically effective quantity to treat the disease or condition.

57. The method of claim 56 wherein the disease or condition is a disease or condition affecting the liver.

58. The method of claim 57 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

59. The method of claim 56 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

60. The method of claim 59 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

61. The method of claim 56 wherein the active plasma protein is selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH), α-1-antitrypsin, and a growth factor.